# Protein folding in a specialized compartment: the endoplasmic reticulum

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The endoplasmic reticulum ensures proper folding of secretory proteins. In this review, we summarize and discuss the functions of different classes of folding mediators in the secretory pathway and propose updated models of the quality control system.

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## Introduction

In all cellular compartments of a eukaryotic cell where protein folding occurs, there is a cohort of proteins, the molecular chaperones, which assist proteins to fold [1]. The Sec61 protein complex translocates secretory and membrane proteins into the lumen of the endoplasmic reticulum (ER), where they attain their correct three-dimensional structure. During this period, proteins undergo post-translational modification. As secretory proteins are folded during their transit through the ER, this compartment can be considered as a specialized location for protein folding.

## The classical chaperones

Molecular chaperones are proteins that bind to unfolded or misfolded proteins in a transient or more permanent manner; it is believed that they facilitate folding and prevent aggregation. Chaperones are not confined to specific compartments, but are distributed over all compartments of the cell. Each compartment has its own set of chaperones, however.

### *BiP/GRP78*

One of the most abundant proteins of the ER is BiP (Table 1) — a member of the Hsp70 family of chaperones [2] that transiently associates with a wide variety of newly synthesized secretory proteins, and more permanently with misfolded or unassembled proteins.

BiP binds to extended stretches of hydrophobic polypeptides [3]. The recent determination of the three-dimensional structure of the peptide-binding domain of the bacterial BiP homolog DnaK with a bound peptide revealed a site accommodating only five amino acid residues with three central hydrophobic sidechains [4]. Sequence comparisons of DnaK and BiP suggest that they have very similar binding sites [5]. The surface charge distribution is predicted to be positive on one side of the binding pocket of BiP, whereas it is negative on both sides in DnaK [5]. This may account for some differences in the specificity of the two chaperones.

The binding of peptides to, and release from, BiP are coupled to the binding and hydrolysis of ATP [6]. This cycle of ATP hydrolysis coupled binding and release is best understood for DnaK [7], where unfolded proteins are delivered to the ATP-bound DnaK by another chaperone, DnaJ, which also stimulates the ATPase activity of DnaK. The release of ADP is rate limiting in the cycle of DnaK and another protein, GrpE, acts as a catalyst of nucleotide exchange. No homolog of GrpE has been found in the ER of eukaryotic cells, but the rate-limiting step of the BiP cycle may be the hydrolysis of ATP, as demonstrated in the case of the cytosolic chaperone Hsc70; the cytosol is also devoid of GrpE [8].

In the ER of yeast, a protein from the peptide translocation machinery, namely Sec63p, has a domain that is homologous to DnaJ, and interaction between this region and BiP is required for the translocation of newly synthesized proteins [9]. The interaction between Sec63p and BiP is not only required for the completion of the translocation process [10], but also for release from the translocon, a subcomplex which engages secretory precursors on the membrane of the ER [11]. At the later stage of translocation, BiP may act as a molecular ratchet directing the translocating polypeptide towards the lumen [12] or as a motor that pulls the translocating polypeptide chain [13].

A role of BiP in protein folding was initially inferred from its binding properties and from the fact that its expression is induced by the accumulation of unfolded proteins in the ER [1]. It has been difficult, however, to establish the real function of BiP *in vivo*. Upon overexpression, BiP selectively retained a subset of secretory proteins (i.e. von

**Table 1**

**Classes of chaperones found in the ER of eukaryotic cells.**

| Chaperone | Family | Species | Protein name |
|---|---|---|---|
| Classical chaperones | GRP78/BiP | Mammalian | GRP78/BiP |
| | | *S. cerevisiae* | Kar2p |
| | | *S. pombe* | BiP |
| | GRP94 | Mammalian | GRP94/gp96 |
| | | *S. cerevisiae* | – |
| | | *S. pombe* | – |
| Lectin-like proteins | Calnexin/calreticulin | Mammalian | Calnexin<br>Calreticulin |
| | | *S. cerevisiae* | Cne1p |
| | | *S. pombe* | Cnx1 |
| Protein disulfide isomerases | PDI | Mammalian | PDI |
| | | *S. cerevisiae* | Pdi1p; Eug1p |
| | | *S. pombe* | SPAC1F5.02 |
| | PDI-like | Mammalian | ERp57/Q-2/ER60/ERp60/ERp61<br>ERp72/CaBP2; P5/CaBP1; PDIR |
| | | *S. cerevisiae* | Yia5p; Mpd1p; Mpd2p |
| | | *S. pombe* | SPAC17H9.14c; SPAC13F5.05 |
| Peptidyl prolyl isomerases | FK506-binding proteins | Human | FKBP13, FKB65 |
| | | *S. cerevisiae* | Fpr2p |
| | | *S. pombe* | SPBC24E9.17C; FKBP39P; SPAC27F1.06C |
| | Cyclophilins | Human | Cyclophilin B; cyclophilin C; cyclophilin 40;<br>cyclophilin 33A; cyclophilin 33B |
| | | *S. cerevisiae* | Cyp2p, Cyp5p; Cpr7p; Cpr8p; Scc3p |
| | | *S. pombe* | Ppi1; Wis2; Cyp1; SPAC57A10.03; SPCC553.04<br>SPAC21E11.05C; SPBC16H5.05C |
| Glucosyltransferases | UDP-glucose: glycoprotein glucosyltransferase | Mammalian | UDP-glucose glycoprotein:glucosyltransferase |
| | | *S. cerevisiae* | – |
| | | *S. pombe* | Gpt1 |

Willebrand factor, but not factor VIII) in the ER in CHO cells [14]. In contrast, using smaller proteins as reporters of secretion, it was found that overexpression of BiP in yeast did not affect secretion, but that lower BiP levels decreased secretion [15]. These observations of a positive role for BiP on protein secretion would be consistent with BiP favoring folding by preventing aggregation, the classical molecular chaperone function. The indication that BiP indeed favors folding in the ER comes from experiments in *Saccharomyces cerevisiae*.

*S. cerevisiae* contains a protein homologous to BiP, namely Kar2p (Table 1), which was originally isolated as a karyogamy gene and is essential for viability [16]. By using temperature-sensitive alleles of *KAR2* containing mutations involved in translocation, it was possible experimentally to separate the translocation and protein folding functions of Kar2p [17], providing evidence that Kar2p can act as a molecular chaperone following translocation.

### *GRP94*

Another chaperone of the ER is the very abundant glycoprotein GRP94 (Table 1), which belongs to the Hsp90 family. Despite its abundance, it is the least understood of the ER chaperones. Like BiP, GRP94 has been shown to associate transiently with a number of peptides and unfolded proteins, including unassembled immunoglobulin light and heavy chains [18]. Stress and the accumulation of unfolded proteins in the ER [19] further induce the expression of GRP94, like that of BiP. Therefore, GRP94 is thought to have a similar function to that of BiP. The mechanism of GRP94 function appears to differ from that of BiP, however, in that GRP94 has been reported to display substoichiometric ATP binding and to possess quite weak ATPase activity [20–22]. Similar observations were reported for the cytosolic paralog, Hsp90 [20]. GRP94 has gained some interest in the field of immunology and cancer research, since the finding that GRP94 is involved in peptide binding and transport for antigen presentation (for a review see [23]).

## Lectin-like proteins

### *Calnexin and calreticulin*

The ER is the initiation site of N-linked glycosylation. This modification is highly conserved from yeast to mammalian cells. Oligosaccharides are attached to some asparagine residues in the sequence Asn-X-Ser/Thr of newly synthesized secretory proteins (for reviews see

[24,25]). Some of the sugar residues are in turn trimmed after transfer of the N-linked oligosaccharide to the protein. The model of quality control of glycoprotein folding is able to assign a link between folding and N-glycosylation. Incompletely folded N-glycoproteins, but not non-glycosylated proteins or folded glycoproteins, were bound in the ER to the type I membrane protein calnexin. Calreticulin (Table 1), a soluble luminal homolog of calnexin, was later also shown to bind similarly unfolded glycoproteins [26].

In the literature, there are reports that calnexin binds polypeptides independently of glycosylation. Association with calnexin (revealed by coimmunoprecipitation) was not abrogated when glycosylation of the substrate was prevented either by inhibitory drugs or by mutation of the glycosylation sites [27,28]. The size of the complex, however, indicated that large aggregates were formed [29]. Moreover, cleavage of the oligosaccharides from a substrate coimmunoprecipitated with calnexin did not result in release from calnexin [27,30]. The substrates selected *in vivo* by immunoprecipitation are by definition unfolded proteins and these are unlikely to be soluble, even after release from calnexin. Thus, the current evidence points to the function of calnexin and calreticulin as lectins.

In *S. cerevisiae*, Cne1p (Table 1) displays significant homology to mammalian calnexin and calreticulin [31]. Cne1p is a type I transmembrane protein that is not essential for viability [32], but is required for normal cell-wall formation [33,34]. Cne1p might function in analogy to mammalian calnexin/calreticulin in quality control, as in Cne1p-deficient (*Δcne1*) cells there was an increase in the cell-surface expression of an ER-retained temperature-sensitive mutant of the α-pheromone receptor, Ste2-3p, and also an increase in the secretion of heterologously expressed mammalian α1-antitrypsin [32].

In *Schizosaccharomyces pombe* the gene locus (Table 1) with significant homology to calnexin/calreticulin is the essential *Cnx1* gene [35,36] coding for a type I transmembrane protein. The transcription of the *Cnx1* gene is upregulated under conditions that lead to the accumulation of misfolded proteins in the lumen of the ER [35,36], suggesting a central role of the *Cnx1* gene in dealing with cellular stress conditions.

To date, neither the biological functions nor the oligosaccharide-binding specificity of either Cne1p in *S. cerevisiae* or the Cnx1 protein in *S. pombe* have been elucidated.

## Protein disulfide isomerases

The formation of disulfide bonds is an important protein modification in the reducing environment of the ER lumen. Disulfide bonds stabilize the native conformation of many secretory proteins. Some proteins are unfolded in the absence of their correct disulfide bonds, in which case folding and disulfide-bond formation are coupled; other proteins can fold without their disulfide bonds, but they confer additional stability. The formation and the rearrangement of disulfide bonds is often rate limiting during folding *in vitro*, and enzymes that catalyze these reactions are considered as genuine folding enzymes.

### *Protein disulfide isomerase (PDI)*

Protein disulfide isomerase (PDI; see Table 1) was the first of the folding enzymes to be identified. The function of PDI in yeast was shown to be its isomerase activity [37]. *In vitro* studies showed that PDI catalyzes the formation, rearrangement and breakage of disulfide bonds, depending on the redox conditions (for a review see [38]).

PDI is about 500 residues long and has five domains, which are designated in sequential order **a**, **b**, **b′**, **a′** and **c**. The **a** and **a′** domains, of about 100 residues each, are strongly homologous to thioredoxin, a small thiol-disulfide oxidoreductase. The intervening segments, **b** and **b′**, are homologous to each other. The C-terminal region (**c**) of about 50 residues is highly acidic. The structures of the **a** and **b** domains have been solved by nuclear magnetic resonance (NMR). Not surprisingly the **a** domain was found to have the same fold as thioredoxin, but more interestingly the **b** domain also has a similar fold, despite the lack of detectable sequence homology with thioredoxin [39].

The mechanism of PDI is not yet fully understood. Each of the thioredoxin domains, **a** and **a′**, contains two active-site cysteine residues, in the sequence Cys-Gly-His-Cys, which are involved in thiol-disulfide exchange reactions. However, although the individual **a** and **a′** domains retain their thiol-disulfide exchange activity, in that they catalyze the introduction of disulfide bonds into substrate proteins, they lack the isomerase activity of the whole enzyme [40]. This finding suggests that faster thiol-disulfide exchanges are not sufficient to account for the full activity of PDI. Catalysis of disulfide isomerization also requires protein–protein interactions, which stabilize non-native conformations of the substrate. Transient unfolding is certainly required to allow the rearrangements of some buried and stable disulfide bonds that are catalyzed by PDI, as observed during the folding of the bovine pancreatic trypsin inhibitor [40].

It has now been shown that full isomerase activity is restored only by the addition, to the catalytic **a** and **a′** domains, of the **b** and **b′** domains, the latter domain being particularly important [41]. The importance of the protein–protein interactions for the isomerase activity of PDI is supported by the fact that the **b′** domain, which is the most important for this activity, is also the domain with the greatest peptide-binding activity [42].

By screening a peptide library it was possible to determine the binding preferences of human PDI. There was a preference towards sequences containing a helix breaker or small residue at the first position, a basic residue at the second, and a hydrophobic residue at the third [43]. Interestingly, much the same substrate specificity was also found in another ER chaperone, the heat-shock protein BiP [3].

The reaction mechanism of yeast PDI (Pdi1p) appears to be the same as for mammalian PDI [44]. Eug1p (Table 1) can complement the function of Pdi1p [45]. At least one of the two genes is required for viability; the double mutant *Δpdi1 Δeug1* is not viable [45], indicating the importance of disulfide isomerization *in vivo*. Interestingly, the promoter regions of *PDI1* and *EUG1* contain an unfolded protein response element (UPRE) [46]. In the presence of misfolded proteins in the lumen of the ER, the expression of chaperone genes containing UPREs in their promoters are upregulated, thus increasing the level of chaperones in the ER [47].

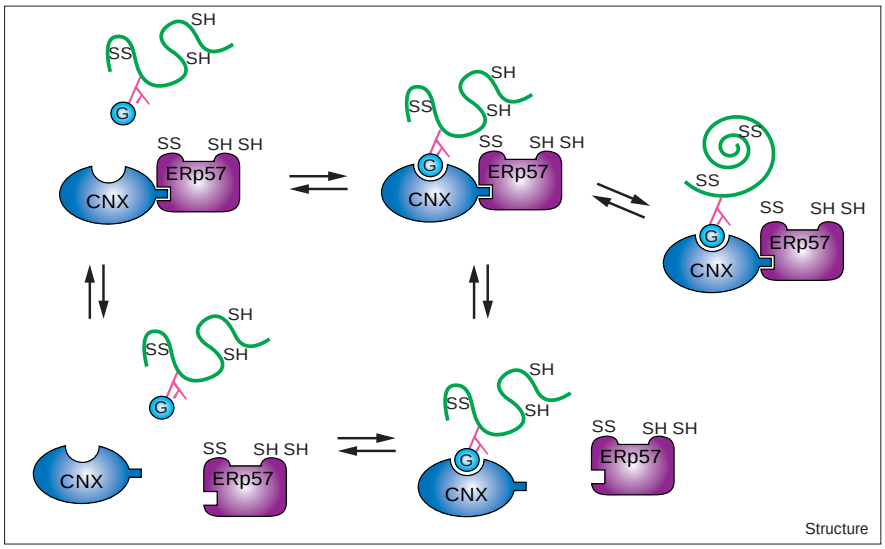
### *PDI-like proteins*

ERp57 [48] (also known as Q-2, ER60, ERp60 and ERp61), and P5 [49] (also known as CaBP1) resemble PDI (Table 1) in size and modular organization — each having two thioredoxin domains. ERp72 [50] (also known as CaBP2) and PDIR (PDI-related protein) [51] are larger proteins with three thioredoxin-like domains. A sixth PDI-like protein was found to be specifically expressed in the pancreas [52]. The functions of these PDI homologs remain elusive, except for that of ERp57.

ERp57 was found to be cross-linked to the same monoglucosylated glycoproteins that are bound to calnexin and calreticulin [53]. *In vitro* experiments revealed that ERp57, like PDI, catalyzes disulfide-bond rearrangements during the refolding of ribonuclease B, but ERp57 does not directly recognize the glycan moiety of its substrate. However, when calnexin or calreticulin were included in the refolding mixture, the catalysis of disulfide-bond rearrangement in monoglucosylated ribonuclease B by ERp57 was greatly enhanced [54]. Thus ERp57 is a PDI which can associate with calnexin or calreticulin and is specialized for folding glycoproteins which are bound to calnexin or calreticulin (Figure 1). It is possible that the function of other PDI-like proteins might similarly rely upon specific interactions with other ER proteins, thus providing a rationale for the multiplicity of PDI-like enzymes in the ER.

The core unit of PDI-like proteins is a thioredoxin-like domain. In addition to *PDI1* and *EUG1*, *S. cerevisiae* contains other genes with potential PDI-like properties (Table 1), such as the loci *YIA5*, *MPD1* and *MPD2*. However, another gene required for disulfide-bond formation, *ERO1*, has also been identified in *S. cerevisiae* [55,56]. At non-permissive temperature, proteins accumulate without disulfide bonds in the ER of temperature-sensitive mutant strains for *ERO1*. Overexpression of Ero1p confers resistance to dithiothreitol, whereas mutation of *ERO1* renders cells hypersensitive to dithiothreitol. It has been proposed that glutathione may only serve as a redox buffer, possibly maintained by Ero1p, and that Ero1p can directly oxidize either the secreted proteins or members of the PDI family, which may then act as oxidant. Alternatively, other small disulfide molecules such as cystines may replace oxidized glutathione. On the basis of sequence analysis, it was suggested that the membrane protein Ero1p may contain an iron–sulfur cluster [55,56]. Ero1p may therefore participate in electron transfer reactions other than thiol-disulfide

**Figure 1**




The cooperation of ERp57 and calnexin to catalyze disulfide-bond formation and rearrangements in glycoproteins. The catalytic activity of the disulfide isomerase ERp57 is greater on monoglucosylated glycoproteins in the presence of calnexin. ERp57 does not recognize the substrate directly, but associates dynamically with calnexin (CNX). (This figure was reproduced from [54] with permission.)

exchanges and the oxidant may be a classical cytoplasmic electron acceptor, such as $NAD^+$.

### Peptidyl prolyl isomerases

All the major cellular compartments where protein folding takes place have been found to contain enzymes that catalyze the isomerization of *cis* and *trans* peptide bonds on the N-terminal side of proline residues. The peptidyl prolyl isomerases (PPIases) comprise two main families: the cyclophilins, which bind to cyclosporin, and the FK-binding proteins (FKBPs), which bind to the FK506 compound.

The FK506-binding protein FKBP65 has been identified by cross-linking experiments using newly synthesized tropoelastin in the ER of fetal bovine chondrocytes [57]. FKBP13 has also been found in the mammalian ER, and contains an ER retention signal [58]. The expression of the *S. cerevisiae* homolog (*FPR1*, FKBP proline rotamase 1) was shown to be upregulated by the accumulation of unfolded protein in the ER, indicating a role for FKBP13 in protein folding *in vivo* [59].

Several cyclophilins have been identified with an N-terminal signal sequence. The involvement of at least one cyclophilin in protein folding in the ER was suggested by the observation that the cyclophilin inhibitor cyclosporin A inhibits partially the triple-helix formation of procollagen *in vivo* [60]. On the basis of sequence similarities, the cyclophilins of the secretory pathway have been classified into several families: CypB [61], CypC [62], CypD [63] and the ninaA-type cyclophilins.

The ninaA cyclophilin from the photoreceptor cells of the *Drosophila melanogaster* eye defines a family of cyclophilins that are type I membrane proteins with a luminal N-terminal cyclophilin sequence. A cyclophilin with a similar organization, Ssc3p, has been found in yeast [64]. NinaA provides an example of a cyclophilin with a narrow substrate specificity, as it is required for maintaining the functional levels of rhodopsins 1 and 2, but not of rhodopsins 3 and 4 [65]. Whether all ER cyclophilins have a narrow specificity and are dedicated to the folding of a few secreted proteins, or whether some serve a general purpose, remains to be established.

### Quality control of glycoprotein folding

Oligosaccharides of newly formed glycoproteins undergo trimming by glycosidases. Glucosidase I acts first to cleave one glucose residue; glucosidase II then removes the two remaining glucose residues to leave the $Man_9GlcNAc_2$ core oligosaccharide [24].

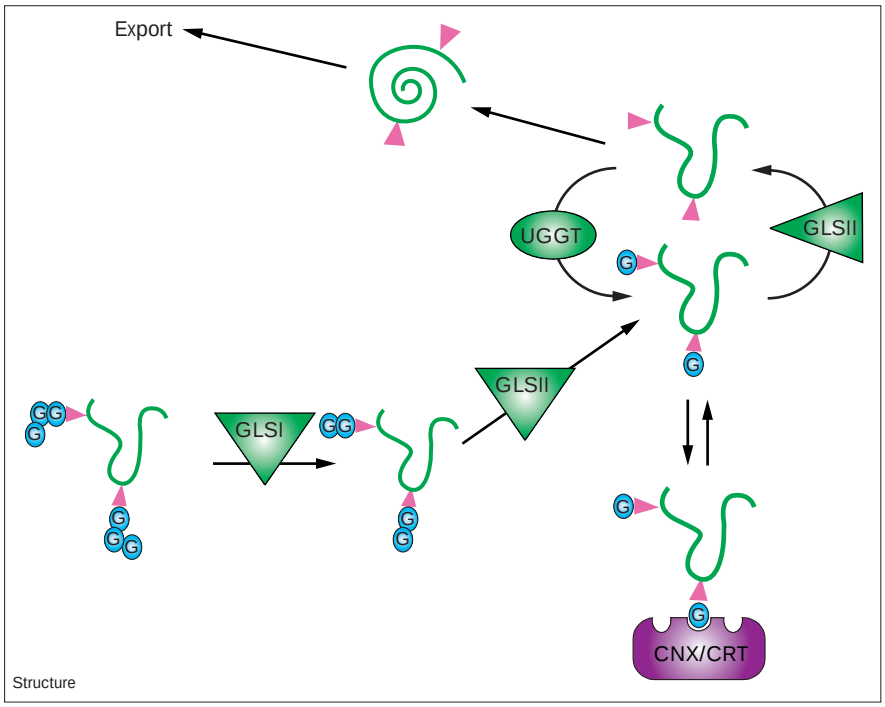
#### *Mammalian cells*

The lumen of the ER of mammalian cells contains a sensor for the folding state of glycoproteins, the UDP-glucose:glycoprotein glucosyltransferase (UGGT), which discriminates between folded and unfolded substrates [66]. UGGT adds back the innermost α1,3-linked glucose residue and thereby tags the incorrectly folded glycoprotein. This monoglucosylated oligosaccharide is a substrate for binding to calnexin and calreticulin that retain the misfolded glycoprotein in the folding environment of the ER lumen. Glucosidase II removes the glucose residue on the oligosaccharide and thus releases the bound glycoprotein. Unfolded glycoproteins are thought to undergo numerous cycles of binding to and release from calnexin and calreticulin. Glucosidase II does not recognize the conformation of the polypeptide, but indiscriminately removes the glucose residues that are present [67]. If the glycoprotein is folded, it is not reglucosylated and escapes from the cycle. If the glycoprotein is unfolded, it is reglucosylated by UGGT and re-enters the cycle [68].

Hammond, Braakman and Helenius [68] first postulated this quality control model for glycoprotein folding (Figure 2). Several lines of evidence support this model. The existence of this cycle was best indicated by the consumption of UDP-glucose for the folding of transferrin in microsomes [69]. The specificity of UGGT was shown not to arise from a greater accessibility of the acceptor oligosaccharides in unfolded proteins, but rather from hydrophobic patches, similar to classical chaperones [70]. The known specificity of UGGT for unfolded proteins, and the *in vivo* abrogation of the binding to calnexin by inhibitors of glucosidase II, led to the formulation of a model whereby only monoglucosylated glycoproteins bind to calnexin or calreticulin [68]. This model is supported by studies with the well-characterized model viral protein influenza hemagglutinin [71] and VSV G protein [68], and more recently from *in vitro* experiments with purified components [54,67]. The ensemble of calnexin, calreticulin, UGGT and glucosidase II can be considered as a molecular chaperone apparatus as their interplay results in the binding and release of unfolded proteins. However, calnexin and calreticulin, the binding components of the system, do not recognize the conformation of their substrates, but act solely as lectins that are specific for the products of UGGT, the enzyme which can discriminate between folded and unfolded glycoproteins [67,72].

It is known that short-lived and misfolded ER or secretory proteins are degraded in a proteasome-dependent pathway in the cytosol. A possible link between quality control and degradation of glycoproteins has been shown by work performed using mannosidase inhibitors. The degradation of the secretion-incompetent α1-antitrypsin variant null (Hong Kong) in mouse hepatoma cells was blocked by deoxymannojirimycin, a specific mannosidase inhibitor [73]. Moreover, the proteasome-dependent degradation of the T-cell receptor subunit CD3-δ in lymphocytes was abrogated with the same mannosidase

**Figure 2**




Model for ER quality control suggested by Hammond, Braakman and Helenius [68]. During the calnexin cycle, newly synthesized glycoproteins are de-glucosylated by the sequential actions of glucosidase I (GLSI) and II (GLSII), making a monoglucosylated protein available for binding to the lectin-like chaperones calnexin and calreticulin (CNX/CRT). Upon dissociation, glucosidase II removes the last glucose residue wherein the protein, if incompletely folded, is subjected to a further round of monoglucosylation by UDP-glucose:glycoprotein glucosyltransferase (UGGT). If fully folded, the glycoprotein is not recognized by UGGT and is allowed to leave the ER (export). Glucose residues in newly synthesized glycoproteins are indicated (G, blue). The pink triangles represent the core oligosaccharide, $Man_9GlcNAc_2$.

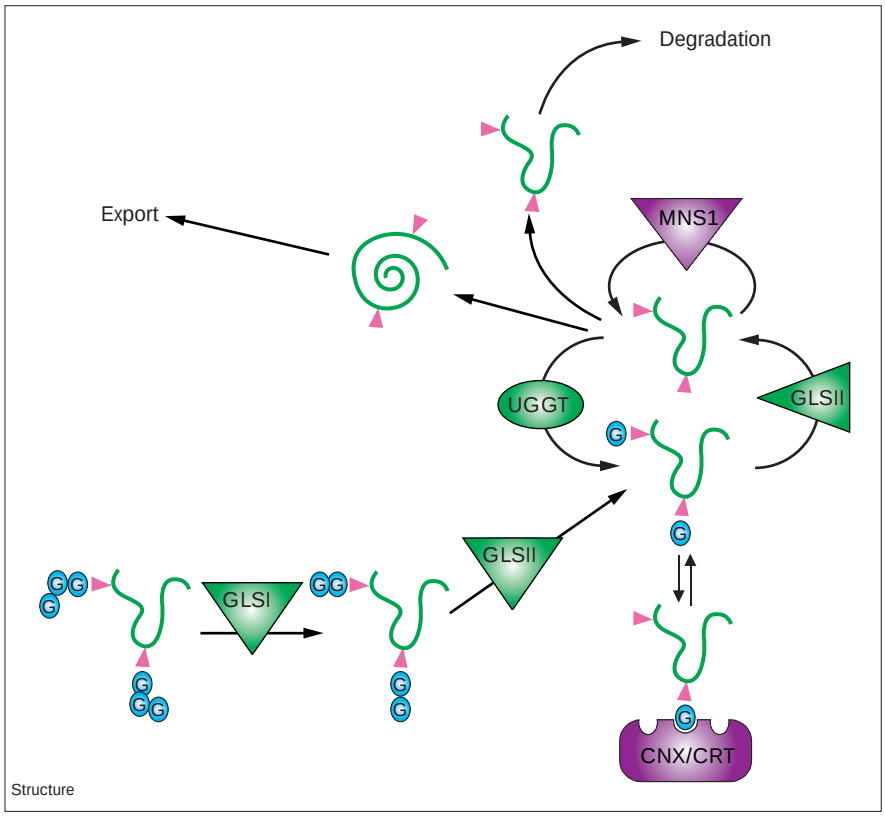
inhibitor [74]. Furthermore, recent work in *S. cerevisiae* indicated the importance of a specific oligosaccharide structure for efficient degradation of a mutated allele of carboxypeptidase Y [75,76]. These results suggested that the mannose residues of the N-linked oligosaccharides and mannosidases might have an important role in the quality control of glycoprotein folding (Figure 3). Glycoproteins that fold correctly exit the ER lumen towards the Golgi apparatus, even if their oligosaccharides have not been trimmed correctly. Cells deficient of glucosidase II or incubated in the presence of glucosidase inhibitors, secrete proteins normally, indicating that trimming is not required for export [71,77]. However, glycoproteins retained in the ER by the UGGT-calnexin-glucosidase II cycle, due to misfolding, are subject to complete trimming by mannosidases. Biochemical evidence suggests that a specific ER-resident α1,2-mannosidase exists in mammalian cells that shows similar activity to Mns1p of *S. cerevisiae* [78]. The removal of the middle α1,2-linked mannose might generate a signal for degradation that is recognized by a lectin-like receptor, which targets the misfolded protein to retrotranslocation and finally to degradation. Hence, the removal of specific mannose residues terminates the time frame set for protein folding [79]. The importance of mannose residues for quality control is supported by the experimental findings that calnexin and calreticulin preferentially bind oligosaccharides of the structure $Glc_1Man_9GlcNAc_2$ [80] and UGGT glucosylates oligosaccharides of the structure $Man_9GlcNAc_2$ [67].

### *Schizosaccharomyces pombe*

*S. pombe* possesses practically the same set of enzymes and proteins involved in quality control as do higher eukaryotic cells. This suggests that the same principle of quality control applies, but there are small modifications to the system as detailed below. UGGT was purified from *S. pombe* and characterized biochemically [81]. The UGGT gene in *S. pombe* was reported to be a stress-inducible gene, as heat shock, reducing agents and calcium ionophores caused an increase in specific mRNA levels [82]. The stress-inducible element in the UGGT promoter, however, no longer exists in higher eukaryotes, such as *D. melanogaster* or rat. *In vitro*, UGGT of *S. pombe* was found to be more stringent in differential glucosylation of denatured and native glycoproteins as compared to rat liver UGGT [81]. Investigating the glucosyltransferase activity *in vivo*, it was reported that the misfolded protein conformation was not sufficient for complete re-glucosylation of all N-linked oligosaccharides [83]. Initial analysis of the N-linked oligosaccharide trimming of *S. pombe* suggested that fission yeast was devoid of a specific ER-luminal α1,2-mannosidase, because the final oligosaccharide-trimming product in the ER lumen was $Man_9GlcNAc_2$ [84]. However, N-linked oligosaccharides of the structure $Man_8GlcNAc_2$ have been reported [83], suggesting that there may be an α1,2-mannosidase in *S. pombe* and hence the mannosidase timer hypothesis might also apply.

**Figure 3**

Extended model for quality control of glycoprotein folding and degradation in mammalian cells. Unfolded or misfolded glycoproteins are subject to folding by chaperones, re-glucosylation by UGGT, retention by calnexin/calreticulin (CNX/CRT) and de-glucosylation by glucosidase II (GLSII). Correctly folded proteins are recognized and exit the ER to subsequent compartments. Upon prolonged presence in the folding environment of the ER, ER-luminal mannosidases (MNS1) further trim mannose residues of the N-linked oligosaccharide in order to generate a degradation signal. This degradation signal is presumably recognized by a receptor with lectin-like properties and targets the glycoprotein to retrotranslocation and degradation by the ubiquitin-proteasome pathway in the cytoplasm. Glucose residues (G) are shown as blue spheres and core oligosaccharides as pink triangles.




*Saccharomyces cerevisiae*

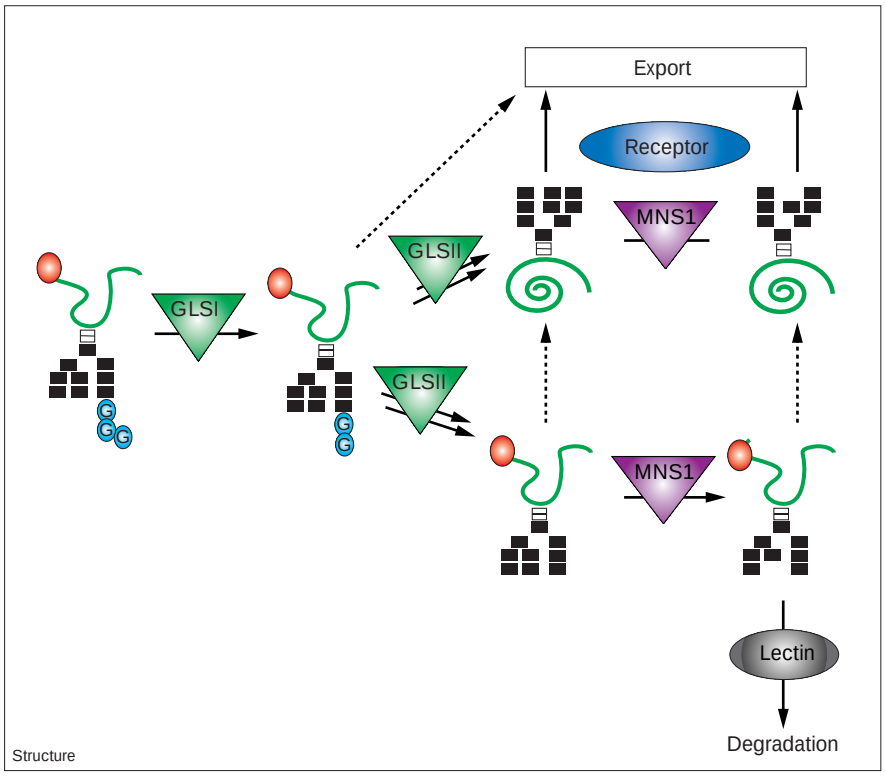
When comparing the components of the quality control system of mammalian cells and *S. cerevisiae*, the most intriguing finding is the apparent absence of UGGT [81]. At the DNA and protein sequence level, *KRE5* displays significant homology to mammalian UGGT [85]; however, to date no evidence for glycoprotein glucosyltransferase activity has been found *in vitro* [81] or *in vivo* [86]. Applying yeast genetics to define specific N-linked oligosaccharides, it was shown that the availability of monoglucosylated N-linked oligosaccharides on glycoproteins reduced the level of unfolded protein in the ER under mild reducing conditions [86]. A quality control system for glycoprotein folding appears to be linear and apparently open in *S. cerevisiae*. Recent data suggest that the number and linkage of mannose residues of an N-linked oligosaccharide influences the degradation rate of misfolded glycoproteins (Figure 4). Yeast strains expressing mutant and thus misfolded carboxypeptidase Y displayed a reduced degradation of this protein when deleted for Mns1p (the ER-luminal α1,2-mannosidase) or for the mannosyltransferases Alg9p and Alg12p. As the removal of the middle α1,2-linked mannose residue occurred at the slowest rate, Mns1p appears to form a timer for degradation of misfolded glycoproteins. Correctly folded glycoproteins are exported to the Golgi apparatus irrespective of their oligosaccharide structure, whereas incorrectly folded glycoproteins remain in the ER lumen until mannosidase I concludes the oligosaccharide trimming. This trimming forms an oligosaccharide structure that is probably recognized by a lectin-like receptor in conjunction with a bound chaperone (i.e. Kar2p). This misfolded glycoprotein will be directed to retrotranslocation and degradation in the cytosol by the proteasome machinery [75].

## Future directions

The ER is a specialized folding compartment, which ensures that only correctly folded molecules proceed further into the secretory pathway and that persistently misfolded proteins are directed to degradation.

Unfolded proteins are bound to classical molecular chaperones and specialized lectins. This association favors protein folding by, as yet, unknown mechanisms. *In vitro* experiments have shown that interaction with molecular chaperones prevents the aggregation of unfolded polypeptides. It has generally been assumed that the same is true *in vivo*. The less limiting definition of chaperone function is that it maintains proteins in a folding competent state. Recent findings, however, suggest that

**Figure 4**




Model for the quality control of glycoprotein folding in *S. cerevisiae*. As opposed to other eukaryotic systems, the system appears to be a linear pathway. Correctly folded proteins can exit the ER even before the N-linked oligosaccharides are completely trimmed. Misfolded glycoproteins remain in the ER lumen and are completely trimmed by glucosidase II (GLSII) and mannosidase 1 (MNS1). The $Man_8GlcNAc_2$ oligosaccharide in conjunction with an associated chaperone (e.g. Kar2p) forms the degradation signal. A receptor with lectin-like properties directs the misfolded protein to retrotranslocation and degradation by the ubiquitin-proteasome pathway in the cytoplasm. N-acetylglucosamine residues (black rectangles), mannose residues (white rectangles) and glucose residues (G, blue) in newly synthesized glycoproteins are indicated. An associated chaperone (e.g. Kar2p) is indicated by the red oval.

additional functions may arise from the cooperation between different ER proteins. Thus, BiP is involved in protein translocation through its interaction with Sec63p [9], and calnexin is involved in disulfide-bond formation by recruiting ERp57 [54]. In the cytoplasm, some PPIases interact with Hsp90, and a similar interaction may occur in the ER between GRP94 and the local PPIases. The global function of the ER results from the cooperation of its many components.

The hypothesis that ER-resident proteins interact loosely with each other to form a dynamic matrix has been around for some time [87], but is difficult to test directly. The observation that several ER proteins can sometimes be coimmunoprecipitated or cross-linked provides only indirect evidence. As most ER-resident proteins are high-capacity low-affinity calcium binders, calcium ions may mediate their association. It has been proposed that this speculative ER protein network may function as a chromatographic matrix for newly synthesized proteins, which would remain adsorbed until losing their affinity by becoming folded [88]. This matrix would be kept in contact with the membrane of the rough ER, where translocation takes place, by transmembrane proteins such as calnexin. In other ER domains, the membrane may not interact with the matrix, so as to allow the accumulation of folded protein ready for export in a budding structure (i.e. ER cargo exit sites). Further research is required to test these hypotheses.

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